Establishment of dexamethasone model as a model for metabolic-associated hepatic injury in male Wistar rats
Ahmed E. Amer¹,²*, Hamdy A. Ghoneim², Rania R. Abdelaziz², George S. G. Shehatou¹,², Ghada M. Suddek²
¹ Department of Pharmacology and Biochemistry, Faculty of Pharmacy, Delta University for Science and Technology, International Coastal Road, Gamasa City, Dakahliya, 11152, Egypt
² Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, 35516, Egypt

*Correspondence: Ahmed E. Amer; Delta University for Science and Technology, International Coastal Road, Gamasa City, Dakahliya, 11152, Egypt; Email, Ph_ahmedamer@yahoo.com; Phone number, +201007844698

ABSTRACT
This study aimed to establish a dexamethasone model as a representative model for metabolic-associated hepatic injury in male Wistar rats. Dexamethasone is a synthetic glucocorticoid with potent anti-inflammatory effects and high glucocorticoid activity. The model was developed to induce insulin resistance and nonalcoholic fatty liver disease in a relatively short period.

Male Wistar rats were allotted into three groups: control, DEX8, and DEX16. The rats of DEX8 and DEX16 groups were injected intraperitoneally with dexamethasone for 6 days at doses of 8 mg/kg/day and 16 mg/kg/day, respectively. Fasting blood glucose levels were measured, and various biochemical parameters were analyzed. Liver sections were collected and examined for histopathological changes.

The results showed that dexamethasone administration significantly increased fasting blood glucose levels compared to the control group. Liver indices were also significantly elevated in the dexamethasone-administered groups. Moreover, serum alanine aminotransferase and aspartate aminotransferase activities were significantly increased in the dexamethasone groups, indicating liver damage. Histopathological examination revealed hydropic degeneration, portal edema, leukocyte infiltration, and fibrosis in the liver sections of dexamethasone-treated rats.

These findings demonstrate that the dexamethasone model successfully induced metabolic-associated hepatic injury in male Wistar rats, as evidenced by hyperglycemia, altered liver indices, increased liver enzymes, and histopathological changes resembling NASH. The model provides a valuable tool for studying the pathophysiology of metabolic liver diseases and evaluating potential therapeutic interventions. It offers a convenient and time-efficient approach to investigate the effects of dexamethasone and develop strategies to mitigate its adverse effects on liver function.

Keywords:
Dexamethasone ; NASH ; Insulin resistance
1. Introduction

Glucocorticoids (GC) are a type of steroid commonly employed for their anti-inflammatory effects in various clinical conditions, including asthma and rheumatoid arthritis. Additionally, GC serve as immunosuppressor in organ transplantation patients. The broad range of clinical applications for GC highlights their significance in managing inflammatory and immune-related disorders (Kumar et al., 2015).

When the body experiences acute stress or a decrease in glucose intake, the endogenous glucocorticoid hormone cortisol is released into the bloodstream. This physiological response is aimed at meeting the body's demand for glucose. Cortisol acts by promoting gluconeogenesis, the process of creating new glucose molecules from non-carbohydrate sources such as amino acids and fatty acids. By increasing blood glucose levels, cortisol ensures an adequate energy supply to meet the body's requirements during times of stress or limited glucose availability (Patel et al., 2011).

Dexamethasone is a frequently prescribed GC that exhibits significantly higher potency in its glucocorticoid activity compared to the naturally occurring cortisol hormone (Du et al., 2015).

Several studies used dexamethasone to induce insulin resistance in whole tissues of rodents (Severino et al., 2002; Patel et al., 2011). Moreover, it was shown that dexamethasone administration to male Wistar rats resulted in the development of nonalcoholic fatty liver disease (NAFLD) (Vinodraj et al., 2015). Furthermore, increased triglycerides (TG) in the liver of dexamethasone-administered rats was reported (Patel et al., 2011; Barbosa et al., 2016). Additionally, it was shown that treating rats with dexamethasone resulted in marked increases in the levels of TG and cholesterol (Mahendran and Devi, 2001). It has been shown that dexamethasone administration resulted in glucose intolerance, hyperglycemia, impaired insulin sensitivity, dyslipidemia, and hepatic steatosis with associated increases in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (El-Sonbaty et al., 2019).

Furthermore, it has been recently shown that severe damage and fatty degeneration in both hepatic and renal tissues was developed by dexamethasone administration, which is combined with significant elevations in serum ALT, AST, alkaline phosphatase (ALP), urea, blood glucose, creatine kinase (CK)-MB, lactate dehydrogenase (LDH), TG, total cholesterol, and oxidative stress (Ahmed et al., 2020). Moreover, Intraperitoneal administration of dexamethasone (4.8 and 16mg/kg) for 6 days developed fatty changes in the livers of experimental rats (Kumar et al., 2015).

In line with this, it was reported that dexamethasone administration resulted in marked lipid accumulation in the liver with elevation of glucose levels, insulin, lipid, liver volume, and liver weight (Vinodraj et al., 2015; Nayak et al., 2017). The main benefit of using dexamethasone models is that they can develop insulin resistance in rodents in a relatively short period (Okumura et al., 1998; Korach-Andre et al., 2005; Kumar et al., 2015).

Taken together, this study was undertaken to establish the model of dexamethasone as described earlier by Kumar et al. (2015) to induce metabolic-associated hepatic injury in Wistar rats.
2. Material and methods

2.1. Drugs

Dexamethasone was utilized as dexamethasone sodium phosphate ampules (Amirya, Egypt).

2.2. Experimental animals

Nine Wistar rats (Males, 250-270 g) were supplied from VACSERA research center (Helwan, Egypt). They were allowed to be accommodated in the Animal House of the Faculty of Pharmacy, Delta University for 10 days before induction of the experiment. They were kept under standard conditions and handled according to the guidelines of the research ethics committee of the Faculty of Pharmacy, Delta University for Science and Technology.

2.3. Experimental design

Following the protocol that has been described early by Kumar et al. (2015), nine male Wistar rats (250-270 g) were allotted into three groups (each of 3 rats), as follows: control group, rats were subjected to injection of saline (16 mg/kg/day, i.p.); DEX8 group, rats were injected with dexamethasone (8 mg/kg/day, i.p.) for 6 days; DEX16 group, rats were injected with dexamethasone (16 mg/kg/day, i.p.). After 6 days of dexamethasone administration, fasting blood glucose was measured, rats were anesthetized by intraperitoneal thiopental (50 mg/kg) (Amer et al., 2021; Amer et al., 2022), blood was collected from the orbital sinus for later biochemical analysis, and rats were sacrificed by decapitation. Later, liver sections were collected, dried, and weighed for determination of liver indices.

2.4. Fasting blood glucose measurement

Rats were subjected to overnight fasting on the 5th day of dexamethasone administration. On the morning of the 6th day of dexamethasone administration, each rat's fasting blood glucose was measured from a blood sample taken by cutting the tail using CareSens® electronic glucometer.

2.5. Assay of serum ALT and AST activities

Serum ALT (Cat.no. 12212, Human diag.) respectively and AST (Cat. No. 12211, Human diag.) activities were determined using commercially available kits according to the supplier’s instructions.

2.6. Histopathology

After dewaxing and hydration, 5 μm slices of left lobes of hepatic tissue were stained with H&E and evaluated by a professional pathologist under a light microscope to detect any pathological changes.
2.7. *Statistical analysis*

Mean ± standard deviation was used to demonstrate the data. Data was analyzed occupying one-way analysis of variance (ANOVA) and Tukey post hoc test. Statistical significance is typically determined by the probability value (P value) of less than 0.05.

3. *Results*

3.1. *Fasting blood glucose levels*

Compared to the control group, DEX8 and DEX16 groups showed significantly higher fasting blood glucose levels by 2.62 and 2.74 folds, respectively (P<0.05, **Figure 1**).

![Figure 1. Impact of dexamethasone (8 and 16 mg/kg/day) on fasting blood glucose](image)

Data are displayed as Mean ± SD. *significant from the control group at P < 0.05, using one way ANOVA.
3.2. **Liver indices**

Relative to the control group, the administration of dexamethasone (8 and 16 mg/kg/day) resulted in a marked increase in liver indices by 2.10 and 2.13 folds ($P < 0.01$), respectively (Figure 2).

![Liver index chart]

**Figure 2. Impact of dexamethasone (8 and 16 mg/kg/day) on liver indices**

Data are displayed as Mean ± SD. *significant from the control group at $P < 0.05$, using way ANOVA.
3.3. Serum ALT and AST

DEX8 group showed considerable raises in serum ALT and AST activities by 3.80 \( (P < 0.05) \) and 3.30 \( (P < 0.01) \), respectively, relative to the control group (Figure 3A and B). Moreover, injection of dexamethasone 16 mg/kg/day resulted in marked elevations of serum ALT and AST activities by 4.58 and 3.44 folds, respectively, relative to the control rats \( (P < 0.01, \text{Figure 3A and B}). \)

Figure 3. Impact of dexamethasone (8 and 16 mg/kg/day) on serum ALT and AST activities

A. Serum ALT activity, B. Serum AST activity. Data are displayed as Mean ± SD. *significant from the control group at \( P < 0.05 \), using way ANOVA. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase.
3.4. *Morphological and histopathological changes*

*Figure 4* demonstrates representative H&E-stained sections from the different groups. Sections from the control group showed normally organized hepatic cords, normal portal area (PA), normal central veins (CV), and normal sinusoids (S). Liver sections from DEX8 group exhibited multifocal areas of hydropic degeneration (black arrowheads) in hepatocytes, congested central veins (red arrows), portal edema with few leukocytes cells infiltration (black arrows), portal fibrosis (yellow arrows) with occluded sinusoids. Liver sections from DEX16 group showed increased hydropic degeneration (black arrowheads) to be diffuse in hepatocytes, congested portal veins (red arrows), and portal fibrosis (yellow arrows) with occluded sinusoids.

![Figure 4: Impact of dexamethasone (8 and 16 mg/kg/day) on histopathological features](image)

*Figure 4. Impact of dexamethasone (8 and 16 mg/kg/day) on histopathological features*

The section of the control group shows normally organized hepatic cords and normal portal area (PA), central veins (CV), and sinusoids (S) while the section from DEX8 group shows multifocal areas of hydropic degeneration (black arrowheads) in hepatocytes, congested central veins (red arrows), portal edema with few leukocytes cells infiltration (black arrows), portal fibrosis (yellow arrows) with occluded sinusoids. DEX16 group’s section shows increased hydropic degeneration (black arrowheads) to be diffuse in hepatocytes, congested portal veins (red arrows), and portal fibrosis (yellow arrows) with occluded sinusoids. PA, Portal area; CV, central veins; S, Sinusoids
**Discussion**

Dexamethasone is a potent synthetic GC with a high affinity for GC receptors (El-Sonbaty *et al.*, 2019). The mechanism underlines this cortisol action depends on increasing hepatic glucose synthesis (gluconeogenesis) and decreasing peripheral glucose uptake into muscle and adipose tissue (Patel *et al.*, 2011; Kumar *et al.*, 2015). The main drawbacks of using therapeutic GC for a prolonged time are hyperglycemia and insulin resistance, which may finally govern the progression of NAFLD (Du *et al.*, 2015).

In the current study, dexamethasone administration (8 and 16 mg/kg/day) for 6 days resulted in considerable metabolic and hepatic consequences including hyperglycemia, increased liver indexes, and alteration of serum liver function markers. These consequences are in line with the previous findings of Kumar *et al.* (2015). Furthermore, dexamethasone administration showed significant pathological alterations in the histological features of the liver. These alterations include ballooning degenerations, inflammation, and fibrosis, mimicking the nature of nonalcoholic steatohepatitis. Nonalcoholic steatohepatitis is the advanced stage of nonalcoholic fatty liver disease which is strongly correlated to metabolic abnormalities including diabetes mellitus.

Tissue insulin sensitivity may be decreased in several ways in response to GC exposure. Insulin signaling pathways are downregulated by GC. GC inhibit the transcription of IRS-1 in mouse skeletal muscle. Simultaneously, they increase the transcription of two proteins that counter insulin action, protein tyrosine phosphatase 1B (PTP1B) and p38 mitogen-activated protein kinase (Almon *et al.*, 2005). Moreover, GC inhibit IRS-1 phosphorylation in the liver and reduce IRS-1 and IRS-2 levels in adipose tissue (Bazuine *et al.*, 2004; Rahimi *et al.*, 2020). Also, GC stimulate the transcription of genes encoding gluconeogenic enzymes such as G6Pase and phosphoenolpyruvate carboxy kinase (PEPCK) to enhance gluconeogenesis in the liver and kidney (Lochhead *et al.*, 2000; Zhang *et al.*, 2019). Moreover, both hyperglycemia and hyperinsulinemia, which are induced by GC, stimulate the activation of sterol regulatory element-binding protein 1c (SREBP-1c) and carbohydrate response element-binding protein (ChREBP). SREBP-1c and ChREBP are responsible for *de novo* fatty acids synthesis (Ferre and Foufelle, 2010; Fillhoulaud *et al.*, 2013; Rahimi *et al.*, 2020).

Furthermore, lipolysis in subcutaneous adipose tissue is stimulated by GC while they induce hypertrophy and differentiation of adipocytes in visceral adipose tissue, in line with the phenotype of Cushing's syndrome patients (Shibli-Rahhal *et al.*, 2006; Vagopoulos and Herzig, 2007; Chanson and Salenave, 2010). GC actions on lipolysis lead to increased levels of released free fatty acids (FFAs) into circulation (Rahimi *et al.*, 2020).

FFAs are taken by tissues such as the heart, skeletal muscle, and liver by fatty acid transporters (Alves-Bezerra and Cohen, 2017). CD36, a well-studied fatty acid transporter, is expressed at a higher level in the liver of GC-treated rats (D’souza *et al.*, 2012). FFAs are then converted in the liver to TG and stored as lipid droplets (Dolinsky *et al.*, 2004; Rahimi *et al.*, 2020). GC stimulate VLDL secretion while they inhibit plasma lipoprotein activity (Rahimi *et al.*, 2020).
Taken together, Plasma TG levels are strongly increased by GC as reported previously (Dolinsky et al., 2004; Choi and Ginsberg, 2011). Moreover, GC suppress beta-oxidation of fatty acids as a result of their inhibition of the transcriptional activity of peroxisome proliferator-activated receptor (PPAR) -α (Marino et al., 2016). PPARα is a key mediator of the expression of mitochondrial acyl-CoA dehydrogenases, which are responsible for the beta-oxidation of FFAs (Vega et al., 2000; Leone et al., 2005). Collectively, GCs-stimulation of lipolysis in the presence of elevated plasma levels of FFAs and stimulation of FFAs uptake by the liver is the predominant mechanism of hepatic lipid accumulation (Rahimi et al., 2020).

Conclusion

In conclusion, the current study observed significant metabolic and hepatic consequences following the administration of dexamethasone in rats. These consequences included hyperglycemia, increased liver indices, and alterations in serum liver function parameters, consistent with previous findings. Furthermore, the histological analysis revealed pathological changes in the liver, resembling features of nonalcoholic steatohepatitis. However, no appreciable changes were observed between the dose of 8 mg/kg/day and 16 mg/kg/day of dexamethasone. Therefore, the dose of 8 mg/kg/day will be appropriate for the induction of these consequences to study the potential therapeutic options in future studies. Moreover, we reviewed some of the possible potential mechanisms that may underlie the hepatic consequences of dexamethasone which may guide possible mechanistic targets of future studies.

Disclosure

The authors declare no conflict of interest.
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